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Study of Total Phenolic Content and Total Flavonoid Content of *Barleria prionitis*

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A B S T R A C T

In recent years phytochemical analysis of plant products took a distinct place in organic chemistry as well as plant biochemistry. One of the bigger challenges of phytochemistry is to carry out all the above operations on vanishingly small amounts of material. Frequently, say that the plant growth regulation, in the biochemistry of plant-animal interactions, or in understanding the origin of fossil plants depends on identifying a range of complex chemical structures which may only be available in a very small amount for study. The present study investigates the qualitative and quantitative analysis of the major phytochemicals from leaves of medicinally important plant *B. prionitis*. Three solvent extraction systems i.e., ethanol, methanol, and chloroform were used for this study. in this research work, Total Phenol Content (TPC) and Total Flavonoid Content (TFC) were determined in different extracts. And best results are shown by ethanolic extract of leaves of *B. prionitis*. These results were used in ethnomedicinal properties of plants.

***Key words*:** *Barleria prionitis*, Total phenolic content, Total flavonoid content, Ethnomedicinal phytochemicals

From several thousands of years, plants have been used as traditional medicine. Ethnomedicinal and traditional uses of natural compounds are offered from the beginning of this century, mainly of plant origin established much interest as they are well tested for their efficacy and generally believed to be safe for humans. 70-80% of the world's population used plant-derived medicine. In rural and tribal areas these medicines are effortlessly accessible sources for healthcare purposes. India is the largest producer of medicinal plants therefore it is perfectly recognized as the botanical garden of the world. Plants are often used as the backbone of all life on the Earth and essential resources for human welfare as raw medicine, food, and fuel. According to World Health Organization (WHO), more than 80% of the world's population depends on traditional medicine that is derived from plants for their health care needs. Traditional plants are derived as medicines that have been used in most parts of the world and their use in microbial diseases has attracted the focus of a number of studies [1]. Plant-derived substances have recently become a great interest in their resourceful applications. It has been estimated that plant species of 14-28% are used for medicinal purposes and that 74% of pharmacologically active phytochemicals components were revealed after following up on ethno-medicinal uses of the plants.

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Chemical compounds produced by plants are known as phytochemicals, generally, to help them resist microorganisms i.e., fungi, bacteria and viruses also considered as antimicrobial activity. The name 'plant' comes from Greek φυτόν (phyton). Some phytochemicals have been used as traditional medicine and others as poisons. Phytochemical analysis refers to the extraction, screening, and identification of the medicinally active substances found in plants. Some of the bioactive substances that can be obtained from plants are flavonoids, alkaloids, carotenoids, tannin, antioxidants, and phenolic compounds [2].

Oxidation is one of the most important processes in which free radicals are produced in foods, chemicals, and even in a living system. Free radicals are produced in mitochondrial respiratory chain reactions. Recent developments in biomedical point to the involvement of free radicals are in many diseases. Free radicals act in the body by attacking the unsaturated fatty acids in biomembranes resulting in membrane lipid peroxidation [3]. Thus, advances in our understanding of phytochemistry are directly related to the successful exploitation of known techniques, and the continuing development of new techniques to solve outstanding problems as they appear. *Barleria prionitis* (Sanskrit: Kuranta; Hindi: Katsareya Marathi: Vjradanti) also known as the Porcupine flower, is a species of plant of family Acanthaceae, native to India, Sri Lanka, and Eastern Southern and Central Africa. The members of this family are mostly found in tropical to subtropical forests, especially in damp and marshy places. Phytochemicals are defined in the strictest sense, as chemicals produced by plants.

MATERIALS AND METHODS

In the present study the plant *B. prionitis* was collected in the early hours of morning from around, Arogya Kendra, Bhopal, India. Preparation of different extracts of the leaves of *B. prionitis* is done by successively using a Soxhlet apparatus using various solvents. The extract was concentrated under a vacuum and preserved in a refrigerator for further details.

Extraction and analysis

Extraction was carried out in different solvents such as petroleum ether, chloroform, and Ethanol at 60°C for 8 hours using the Soxhlet extractor. After extraction, the extracts were dried at room temperature until the extract acquires the solid form. Different organic solvents extract of *Barleria prionitis* were used to screen the following phytochemicals like sterol, reducing sugar, alkaloids, phenolic compounds, flavonoids, tannins, saponins, amino acids, glycosides. Phytochemical tests for analysis of phytoconstituents were carried out in extracts using the standard procedures as described by Sofowara [4], Trease and Evans [5] and Harborne [6]. The various extracts of *B. prionitis* were subjected to phytochemical tests to screen the following chemicals. The phytochemical analysis was conducted using the test developed by Kapoor *et al*. [7] and the presence of different phytochemicals in the extract were listed in the table, which would further be used for quantitative investigations. The Antioxidant activity of plant extract was carried out in different solvents.

Estimation of total phenolic content (TPC)

The capacity of total phenolic contents was determined using the method with slight modification. The total phenolic content of isolated crud was determined by the method described by [8]. 1.0 ml of sample was mixed with 1.0 ml of Folin and Ciocalteu's phenol reagent. After 3 min, 1.0 ml of saturated Na_2CO_3 (~35%) was added to 2 3 the mixture and made up to 10 ml by adding distilled water. The reaction was kept in the dark for 90 min, observed under UV-Vis spectrophotometer at 760 nm absorbance. Gallic acid was used as a standard with varied concentrations from 200 ppm to 1000 ppm. A calibration curve was constructed with different concentrations of catechol (0.01- 0.1 mM) as standard. The results were expressed as mg of catechol equivalents/g of extract and the same procedure was done with Extract.

Estimation of total flavonoid content (TFC)

Flavonoids content of isolated crude were determined this method [9]. Take a clean test tube and add 0.5 ml of the sample (Extract) containing 1.25 ml of distilled water. Then added 0.075 ml of 5% sodium nitrite solution and allowed to stand for 5 min. Added 0.15 ml of 10% aluminum chloride, after 6 min 0.5 ml of 1.0 M sodium hydroxide were added and the mixture was diluted with another 0.275 ml of distilled water. The absorbance of the mixture at 510 nm was measured immediately. The flavonoid content was expressed as mg catechins equivalents /g sample and the same procedure was done with extract.

RESULTS AND DISCUSSION

Phytochemical screening

The result of preliminary phytochemical screening tested positive for Flavonoides, Tannic and Phenolic Compounds. The Methanoilic extract had the highest TPC followed by the ethanolic extract and absence of chloroform extract are found in the TPC shown in (Table 3). The result of TFC of the different extract of the *Barleria prionitis* in the term of µg Quercitin equivalence per ml the result shown (Fig 2) exhibit methanolic extract has the highest TFC followed by ethanolic extract and absences of TPC in the chloroform extract are found [10].

Table 1(a) Premilary phytochemical screening with methanol extract

Test name	Result with methanol extract
Carbohydrate	-
Non-reducing polysaccharide	+
Proteins	+
Protein containing sulfur	-
Steroids	+
Glycosides	-
Carbonate	-
Coumarins glycosides	-
Anthroquinone glycosides	-
Flavonoides	+
Alkaloids	+
Tannic and phenolic compounds	+
Organic acid	+
Inorganic acid	-
Chloride	-
Nitrates	-

Table 1(b) Premilary phytochemical screening with ethanol extract

Test name	Result with ethanol extract
Carbohydrate	-
Non-reducing polysaccharide	-
Proteins	-
Protein containing sulfur	-
Steroids	-
Glycosides	-
Carbonate	-
Coumarins glycosides	-
Anthroquinone glycosides	-
Flavonoides	+
Alkaloids	-
Tannic and phenolic compounds	+
Organic acid	+
Inorganic acid	+
Chloride	-
Nitrates	-

Table 1(c) Premilary phytochemical screening with chloroform extract

Test name	Result with ethanol extract
Carbohydrate	-
Non-reducing polysaccharide	+
Proteins	+
Protein containing sulfur	-
Steroids	+
Glycosides	-
Carbonate	-
Coumarins glycosides	-
Anthroquinone glycosides	-
Flavonoides	-
Alkaloids	+
Tannic and phenolic compounds	+
Organic acid	+
Inorganic acid	-

Chloride	-
Nitrates	-

Table 2 Total phenol content

Quantitative analysis	Methanol	Ethanol	Chloroform
Total phenols (µg of GAE/serving)	42.2659±28.68	17.669±33.51	Nil

Table 3 Absorbance of tannic acid and total phenolic contents in extract

S. No.	Concentration (µg/ml)	Absorbance (765 nm)
S-1	20	1.248
S-2	40	1.332
S-3	60	1.439
S-4	80	1.526
S-5	100	1.634

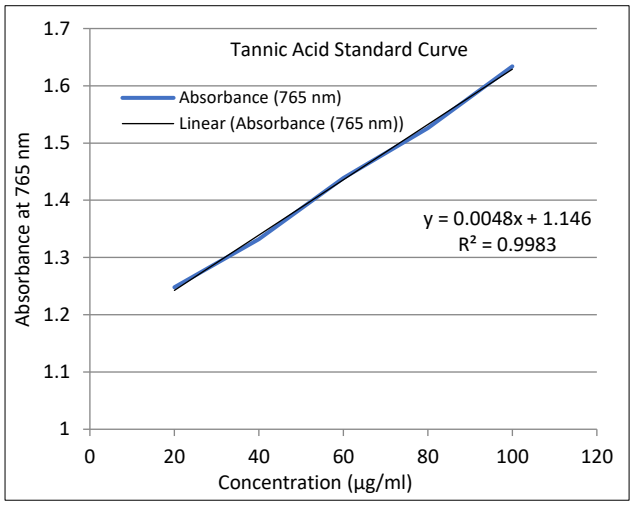

Fig 1 Standard curve of tannic acid

Absorbance of Quercetin for quantification of total flavonoid Content's in *Barleria prionitis* leaf extract.

Table 3 Total Flavonoid content (TFC) assay

S. No.	Concentration µg/ml	Absorbance (510 nm)
S-1	20	0.140
S-2	40	0.199
S-3	60	0.304
S-4	80	0.435
S-5	100	0.496

Table 4 Total flavonoid content

Quantitative analysis	Methanol	Ethanol	Chloroform
Total flavonoids (µg of QE/serving)	56.569±09.60	33.324±03.40	Nil

Under basic reaction conditions a phenol loses a hydrogen ion to produce a phenolate ion, which reduces Folic–Ciocalteu reagent [11-12]. The change is monitored spectrophotometrically. As phenolic (including many Flavonoids) conyain polar phenolic hydroxyl group, their high extraction into methanol and Ethanol is quite reasonable.

Absorbance of Quercetin for quantification of total flavonoid Content's in *Barleria prionitis* leaf Extract indicates that as the concentration increases absorbance also increases. Hence the straight-line equation is obtained. Similarly, Absorbance of Tannic Acid and Total Phenolic Content's in Extract i.e., Tannic acid standard curve also indicates that as the concentration increases absorbance also increases. Hence the straight-line equation is obtained.

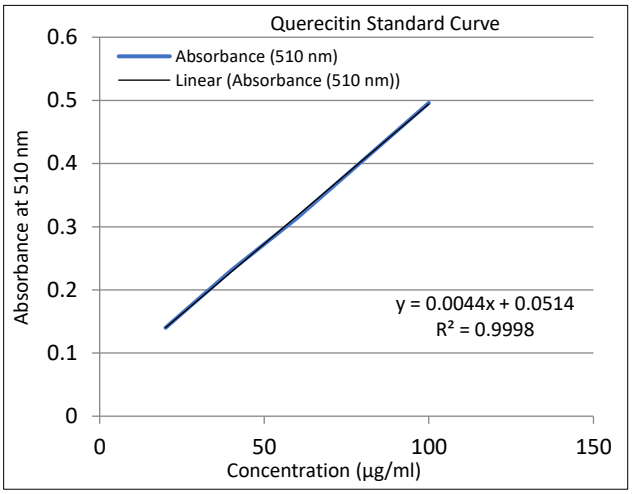

Fig 2 Standard curve of Quercitin

CONCLUSION

The *Barleria prionitis* most often found on India, with the presence of spines it is ignored by cattle's and considered as a weed. In the present work we have been trying to establish the total phenolic content assay and total flavonoid content in three different extracts of leaves of *Barleria prionitis* that is ethanol, methanol and chloroform extract. From these results we understand that the ethanol plant extract gives highest value of TPC and TFC. This can be helpful in establishing of its therapeutic values. The above results have shown the presence of total phenolic contents and total flavonoid contents imply that the ethanolic extract of both the parts was most abundant, as compared to other extracts. From this we can conclude that the phenolic compounds can be better isolated with solvents of higher polarity range. The importance of TPC and TFC it shows medicinal activities like antioxidant properties, diuretic properties, nephrolithiasis and renal calculi.

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